Antibacterial activity of garlic extract on streptomycin-resistant *Staphylococcus aureus* and *Escherichia coli* solely and in synergism with streptomycin

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Abstract

This study focuses the significant antibacterial activity of Garlic (*Allium sativum* Linn.) extract on streptomycin-resistant strains solely and in synergism with streptomycin. Gram-positive *Staphylococcus aureus* ATCC BAA 1026 and gram-negative *Escherichia coli* ATCC 10536 were made resistant to standard antibiotic streptomycin used as a control in the experiment. Zones of inhibition of different treatment groups were measured by agar-well-diffusion assay and compared with control. Statistical comparison of sole extract and streptomycin synergism with streptomycin control had proved it significant.

Key words: Antibacterial, antibiotic, *Escherichia coli*, resistance, *Staphylococcus aureus*, synergism

INTRODUCTION

Since ancient time, naturally occurring plants have played an important role in the discovery of new therapeutic agents.[1] Almost all antibiotics are subjected to the problem of bacterial resistance. Therefore, newer herbal antibacterial compounds from plants and their semisynthetic derivatives to overcome the resistance are under investigation.

Garlic (*Allium sativum* Linn.) has an important dietary and medicinal role for centuries. Its therapeutic uses include beneficial effects on the cardiovascular system, antibiotic, anticancer, anti-inflammatory, hypoglycemic, and hormone-like effects.[2] Garlic extracts have been used to treat infections for thousands of years.[3] Its typical pungent odor and antibacterial activity depend on allicin, which is produced by enzymatic (alliin lyase) hydrolysis of alliin after cutting and crushing of the cloves.[4]

Streptomycin was widely used for more than four decades, which is still effective against gram-positive and gram-negative bacteria. This study reveals better efficacy of garlic extract and its streptomycin synergism than streptomycin on resistant strains.

MATERIALS AND METHODS

Source of bacterial strains

Both gram-positive *Staphylococcus aureus* ATCC BAA 1026 and gram-negative *Escherichia coli* ATCC 10536 were collected from clinical specimens of Padmashree Diagnostic Centre, Tumkur, India.

Development of streptomycin resistance in selected bacterial strains

Each organism was subcultured from a nutrient agar (Qualigens Fine Chemicals, Mumbai, India) slant to standard methods broth (Human Diagnostic and Surgichem, kolkota, India), PH 7.8, and incubated overnight. With stock solutions of standard antibiotic streptomycin (gift sample on request from Karnataka Antibiotics and Pharmaceuticals Limited, Bangalore, India), which were prepared by diluting weighed aliquots of this drug in

sterile 1% phosphate buffer PH 6.0, twofold dilutions were prepared daily. The dilution series were usually consisted of ten 100× 13 mm test tubes each containing 0.5 ml of the antibiotic dilution. To each tube was added 1.5 ml of a 1:100 dilution in broth of the 18–24 h broth culture prepared above, and all the tubes were incubated at 37°C for 24 h. The last tube showing inhibition of the organism in the dilution series indicated the initial sensitivity of the strain in micrograms of the antibiotic. The second tube showing growth in dilution series was selected for preparing 1:100 broth dilutions for the second exposure to streptomycin dilution series. To increase the resistance of the strain to the particular antibiotic, the procedure described above was repeated.[5]

Preparation of aqueous garlic extract

Fresh garlic (*Allium sativum* L.) bulbs were purchased from local market. The bulbs were peeled, weighed (100 gm) and cleaned. Cleaned cloves were surface-sterilized by immersing them into 70% (v/v) ethanol for 60s.[6] Residual ethanol on surface was evaporated in sterile laminar airflow chamber followed by homogenizing aseptically in sterile mortar and pestle. The homogenized mixture was filtered through sterile cheesecloth. This extract was considered as the 100% concentration of the extract. The concentrated mother extract was further diluted to 75% and 50% by mixing with appropriate sterile distilled water.[7]

Testing of antibacterial activity using agar well diffusion method

Resistant bacterial strains were inoculated into 10 ml of sterile nutrient broth, and incubated at 37 °C for 8 h. Each culture was swabbed on the surface of sterile nutrient agar plate in duplicate. In each agar plate of both sets, five wells were prepared with the help of sterilized cork borer of 10 mm diameter. In the wells of first plate of each set, 100 µl test samples of following concentrations: (1) standard streptomycin 10 mg/ml in sterile distilled water; (2) 50% sterile garlic extract; (3) streptomycin 10 mg/ml in 50% sterile garlic extract; (4) streptomycin 10 mg/ml in 75% sterile garlic extract; (5) streptomycin 10 mg/ml in 100% sterile garlic extract) were added by using micropipette. In the wells of second plate of each set, 100 µl test samples of the following concentrations: (1) standard streptomycin 10 mg/ml in sterile distilled water; (2) 50% sterile garlic extract; (3) streptomycin 10 mg/ml in 50% sterile garlic extract; (4) streptomycin 15 mg/ml in 50% sterile garlic extract; (5) streptomycin 20 mg/ml in 50% sterile garlic extract) were added. Every plate used according to the aforementioned procedure was performed in triplicate for statistical average.

RESULTS

Mean zones of inhibition were expressed in mm ± standard error of mean. Mean zones of inhibition of different treatment groups were measured by agar-well-diffusion assay and compared with the control. Statistical comparison of sole garlic extract and streptomycin synergism (same concentration of streptomycin in garlic extract of different strengths and different concentration of streptomycin in the garlic extract of same strength as stated in Tables 1 and 2, respectively) with streptomycin control by one-way ANOVA post-test using the software graphpad Instat 3 (trial) had proved it significant. Figures 1 and 2 illustrate Table 1 whereas Figures 3 and 4 do the same for Table 2.

DISCUSSION

The findings of this study reveal the distinct antibacterial profile of *Allium sativum* Linn. solely and in streptomycin synergism against streptomycin-resistant *S. aureus* ATCC BAA 1026 and *E. coli* ATCC 10536 as witnessed from prominent zones of inhibition. *E. coli* is a common pathogenic bacteria for urinary tract infection and *S. aureus* is the cause of pneumonia and several infections in gut, urinary tract, etc. Use of garlic extract solely is fruitful.

Table 1: Inhibition of resistant bacteria due to sole garlic extract and synergism of same concentration of streptomycin in garlic extract of different strengths in the presence of streptomycin control

Drug	Dose	Zone of inhibition (mm ± S.E.M.)	
		***S. aureus* ATCC BAA 1026**	***E. coli* ATCC 10536**
Streptomycin[a] (control)	10 mg/ml	7 ± 0.2887	8 ± 0.2887
Sterile garlic extract[a]	50%	14 ± 0.5775**	14.5 ± 0.2887**
Streptomycin[b]	10 mg/ml	15 ± 0.2887**	16 ± 0.2887**
Streptomycin[c]	10 mg/ml	22 ± 0.2887**	24 ± 0.5774**
Streptomycin[d]	10 mg/ml	26 ± 0.2887**	28 ± 0.2887**

[a]Solvent: sterile distilled water. [b]Solvent: 50% sterile garlic extract. [c]Solvent: 75% sterile garlic extract. [d]Solvent: 100% sterile garlic extract. $^{**}P < 0.01$ as compared with control according to one-way ANOVA post-test.

Table 2: Inhibition of resistant bacteria due to sole garlic extract and synergism of different concentration of streptomycin in garlic extract of same strength in presence of streptomycin control

Drug	Dose	Zone of inhibition (mm ± S.E.M.)	
		***S. aureus* ATCC BAA 1026**	***E. coli* ATCC 10536**
Streptomycin[a] (control)	10 mg/ml	6 ± 0.2887	7 ± 0.2887
Garlic extract[a]	50%	15 ± 0.2887**	14 ± 0.2887**
Streptomycin[b]	10 mg/ml	16 ± 0.2887**	15 ± 0.2887**
Streptomycin[b]	15 mg/ml	19 ± 0.5774**	17 ± 0.2887**
Streptomycin[b]	20 mg/ml	20 ± 0.2887**	19 ± 0.5774**

[a]Solvent: sterile distilled water. [b]Solvent: 50% sterile garlic extract. $^{**}P < 0.01$ as compared with control according to one-way ANOVA post-test.

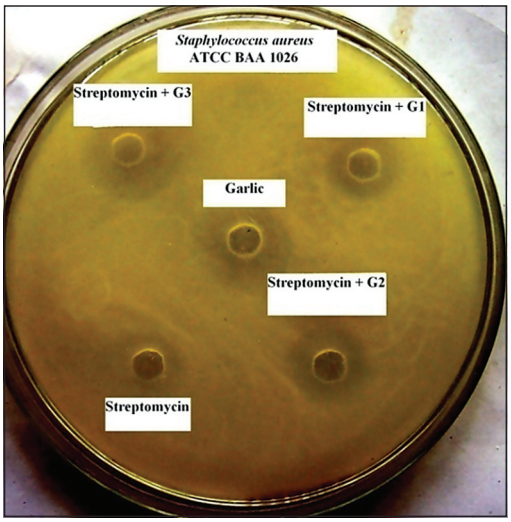

Figure 1: G1: 50% garlic extract; G2: 75% garlic extract; G3; 100% garlic extract. The concentration of streptomycin alone and in G1, G2, G3, etc. is 10 mg/ml. Concentration of garlic used alone is 50%.

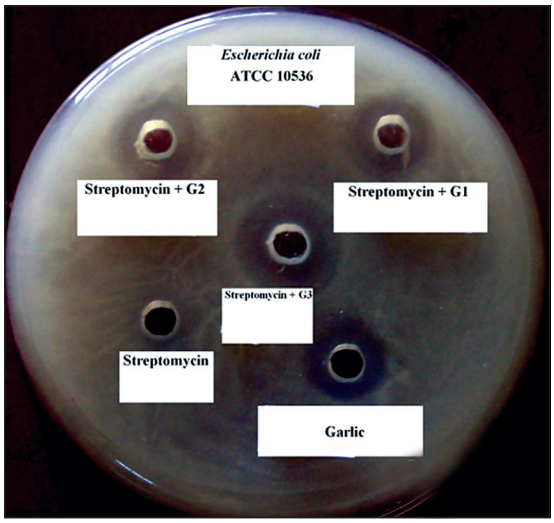

Figure 2: G1: 50% garlic extract; G2: 75% garlic extract; G3: 100% garlic extract. The concentration of streptomycin alone and in G1, G2, G3, etc. is 10 mg/ml. Concentration of garlic used alone is 50%.

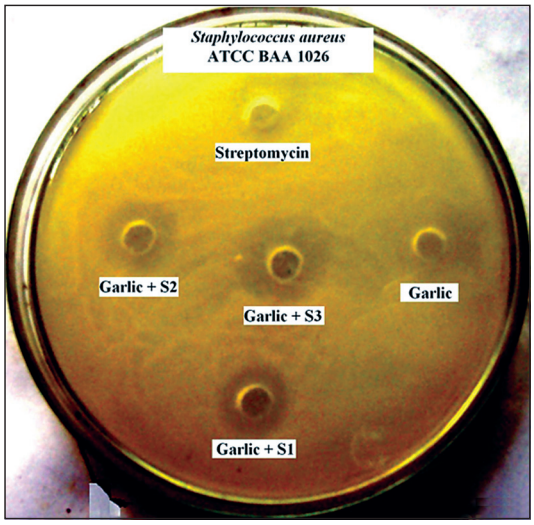

Figure 3: S1: 10 mg/ml; S2: 15 mg/ml; S3: 20 mg/ml of streptomycin. The concentration of garlic extract used alone and with S1, S2, S3, etc. is 50% garlic extract. Concentration of streptomycin used alone is 10 mg/ml.

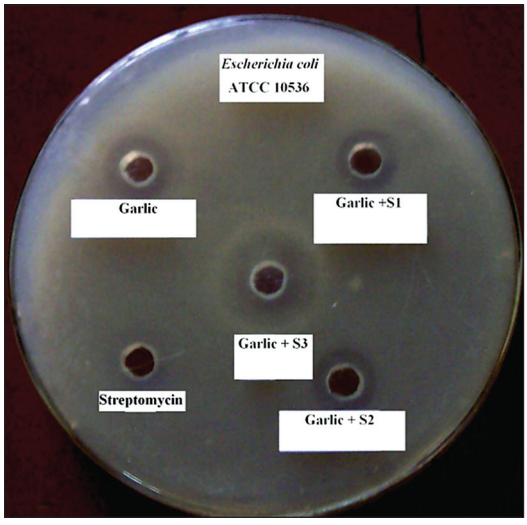

Figure 4: S1: 10 mg/ml; S2: 15 mg/ml; S3: 20 mg/ml of streptomycin. The concentration of garlic extract used alone and with S1, S2, S3, etc. is 50% garlic extract. Concentration of streptomycin used alone is 10 mg/ml.

Synergistic use can prevent the pathogenic organism grow their resistance against antibiotic.

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